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TECHNICAL NOTE

Characterisation and cross-amplification of polymorphic microsatellite loci in ant-associated root-aphids

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Abstract Twenty-six polymorphic microsatellite loci were developed for four species of ant-associated root-aphids: *Geoica utricularia*, *Forda marginata*, *Tetraneura ulmi* and *Anoecia corni*. We found up to 9 alleles per locus, with an average of 4.8. We also report polymorphic cross-amplification of eleven of these markers between different pairs of study species. Furthermore, we tested previously published aphid microsatellites and found one locus developed for *Pemphigus bursarius* to be polymorphic in *G. utricularia.* These microsatellite markers will be useful to study the population structure of aphids associated with the ant *Lasius flavus* and possibly other ants. Such studies are relevant because: 1. *L. flavus* mounds and their associated flora and fauna are often key components in protected temperate grasslands, and 2. *L. flavus* and its diverse community of root-aphids provide an interesting model system for studying the long-term stability of mutualistic interactions.

Keywords Microsatellites · Root-aphids · Mutualism · Aphidoidea (Hemiptera) · Pemphigidae · Anoeciidae

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Mutualistic interactions between species are widespread and play key roles in ecosystem stability and diversity (Stachowicz 2001; Bastolla et al. 2009). In Northwest Europe, the yellow meadow ant *Lasius flavus* keeps up to fourteen species of mutualistic root-aphids in its nests (Pontin 1978; Heie 1980; Godske 1991). The ants actively tend the aphids, which provide them with honeydew (Pontin 1978). The nest mounds are markers of high grassland biodiversity and long-term habitat stability (Dean et al. 1997; Blomqvist et al. 2000; Lenoir 2009). However, despite the decline of European temperate grasslands in recent decades and the associated losses in plant and invertebrate biodiversity (WallisDeVries et al. 2002), neither the sociobiology of the ants (but see Boomsma et al. 1993), nor the biology of the root-aphids (Pontin 1978; Godske 1991, 1992) have been extensively studied. To facilitate molecular ecological approaches in the study of this mutualism, we developed DNA microsatellite markers for the four commonest species: *Forda marginata*, *Tetraneura ulmi*, *Geoica utricularia* and *Anoecia corni*.

Samples for genomic library construction for *Forda marginata*, *Tetraneura ulmi*, and *Anoecia corni* were collected in 2007 from an ant-nest on the Dutch island of Schiermonnikoog (53°29′03.5″N; 6°13′46.1″E) whereas *Geoica utricularia* was collected near Dejret, Denmark (56°12′54.2″N; 10°24′48.2″E). All samples for molecular analysis were preserved in 96% ethanol.

Genomic DNA was extracted using the QIAGEN DNeasy Blood & Tissue kit and enriched for poly-CA and poly-CT microsatellite containing fragments using the protocol by Rütten et al. (2001). We designed PCR primers for the flanking regions of repetitive motifs using the web-based software Primer 3 (Rozen et al. 2000).

Primers were tested on Schiermonnikoog samples collected in 2007, 2008 and 2009 and on samples collected

Table 1 Characteristics of 26 polymorphic microsatellite loci in different species of ant-associated root-aphids

Locus	Species	Primer sequence (5′–3′) (F: forward. R: reverse)	Repeat motif	Size range (bp)	N	N_a	H_E	H_O	T_a (°C)	Nr. of cycles x	Primer concentration (μM)	Multiplex mix	Genbank accession number
Gu1	*Geoica utricularia*	F: ATCAAACGAACGAACCGAAT R: GCGAAAGTTATGGCGTTTGT	$(GT)_8$	113–118	5	4	0.740	1.000	50	40	0.35	Gu-3	HM582813
Gu2	*Geoica utricularia*	F: CGCGATTAGATCTCGGAATG R: AAATCGTATAAAAGTAAAGGCGTTAT	$(GT)_{11}$	158–177	227	5	0.613	0.361	50	40	0.15	Gu-2	HM582814
Gu3	*Geoica utricularia*	F: TATCGTGCGGACACAGACAT R: CGGGCTATACCGCATACACT	$(TA)_9$	192–208	169	7	0.665	1.000	50	40	0.15	Gu-1	HM582815
Gu4	*Geoica utricularia*	F: CTGCTGCTCGTCCGACTTA R: GCAGATAAAAACTGTTAGCCTTGA	$(TG)_6$ C $(AT)_{12}$	206–222	8	4	0.602	0.125	50	35	0.35	Gu-3	HM582816
Gu5	*Geoica utricularia*	F: CACAGGACGCGTAACTTAATATAG R: ACACTTTTCGGCAATTTCGT	$(GT)_{15}$	164–214	214	6	0.569	0.145	50	40	0.15	Gu-2	HM582817
Gu6	*Geoica utricularia*	F: ATCAAACGGTCTGGCATGTA R: CAATATCTCATCTGCCAGCAA	$(TG)_3$ CG $(GT)_8$	151–200	199	7	0.539	0.337	50	40	0.15	Gu-2	HM582818
Gu7	*Geoica utricularia*	F: GTTAAAGGAACTCTTACGCTCTACG R: CATATAAATAAAAACGTCCTGTAGGC	$(CA)_3$ TA $(CA)_5$	87–103	13	4	0.698	0.000	50	40	0.35	Gu-3	HM582819
Gu8	*Geoica utricularia*	F: TATACACGTCCGCGCAGATA R: GTTCGTTGCTCGTCGACTTT	$(AC)_{10}$	233–237	199	3	0.479	0.060	50	40	0.15	Gu-1	HM582820
Gu9	*Geoica utricularia*	F: CGCGCGTTATGAAAAATGTA R: CTCGCTGTGTGTGACACCTT	$(CA)_{13}$	223–250	184	8	0.800	0.799	50	40	0.15	Gu-1	HM582821
Gu10	*Geoica utricularia*	F: CGCCGCTAAAGAAGTTTTCA R: TTACGTTAAACA(AC)ACGAGGATTTAT	$(GT)_{19}$	228–261	14	8	0.763	0.786	50	40	0.35		HM582822
Gu11	*Geoica utricularia*	F: CGGTTACCCGTAAAAGGCTTA R: AAATCGCAATGACAGTCACG	$(CA)_{11}$	145–153	223	6	0.729	0.677	50	40	0.15	Gu-2	HM582823
Gu12	*Geoica utricularia*	F: GAGCCAACTGCCCGTTATAG R: CGGTTTTATTTAAGGTCTCGAA	$(GT)_{12}$ GC $(GT)_{25}$ A $(GT)_4$	106–138	10	3	0.460	0.000	60	45	0.15		HM582824
Gu13	*Geoica utricularia*	F: TCGCCGTCGACTATTTTACA R: AGTTACGTCCGGGGAGAAAT	$(CAG)_7$ $(N)_{21}$ $(TC)_{10}$	202–218	188	7	0.754	1.000	50	40	0.15	Gu-1	HM582825
Gu15	*Geoica utricularia*	F: TTTTTACGGGCTAAACCCTATTT R: CCAATACGGATCCCAACTTTT	$(GA)_{15}$ $(A)_4$ $(GA)_3$ $(A)_9$	165–167	10	2	0.180	0.200	50	40	0.25		HM582826
Fm1	*Forda marginata*	F: CCTCCAATTACCGTTCAACC R: GAAGAACGTGACACGCGATA	$(TG)_{22}$ CG $(TG)_5$	182–259	154	9	0.458	0.253	53	37	0.15		HM582827
Fm3	*Forda marginata*	F: TCTGATTTTTCGTTCGTCCA R: CGCGGCTCGTTACCTATTTA	$(AT)_{10}$	225–349	138	6	0.494	0.246	50	40	0.15		HM582828
Fm4	*Forda marginata*	F: CATTACGTGTGAGTGTAATATAGTTTT R: TGGTTTAAACGACGGATTTTC	$(AC)_{14}$	178–200	162	7	0.465	0.167	50	35	0.15		HM582829

Table 1 continued

Locus	Species	Primer sequence (5′–3′) (F: forward. R: reverse)	Repeat motif	Size range (bp)	*N*	N_a	H_E	H_O	T_a (°C)	Nr. of cycles *x*	Primer concentration (μM)	Multiplex mix	Genbank accession number
Fm6	*Forda marginata*	F: TCACTCGCCTAGCGTTATTC R: GTGGCCGTAGCATGTCACTA	$(T)_{11}$ ATGA $(T)_{23}$	250–280	125	4	0.709	0.920	50	45	0.15		HM582830
Tu1	*Tetraneura ulmi*	F: CGGGTGCGTGGGTACATTAT R: ATACGTTGAGCCAACTACCG	$(GT)_4$ GAT$(AG)_5$ T-$(GA)_{10}$ $(A)_6$ $(N)_6(T)_{17}$	218–241	89	2	0.164	0.000	50	35	0.25		HM582831
Tu2	*Tetraneura ulmi*	F: TCCGACATACGTTTAACCAAAA R: ATGACACCCCTGCCACTATC	$(TA)_7$ $(TG)_8$	157–159	60	2	0.180	0.000	50	40	0.25	Tu-1	HM582832
Tu3	*Tetraneura ulmi*	F: CGCCGTAAATAATAATAACAACAA R: CACGAGACCAAGAGATAAGGAAA	$(A)_{11}$ $(AT)_6$ $(TA)_2$ $(C)_3$ $(GT)_9$	234–264	89	5	0.702	0.921	50	35	0.25		HM582833
Tu4	*Tetraneura ulmi*	F: TTATTCGCAACCACACCTTG R: ACGCGACGGATAGAAATACG	$(GT)_{26}$ G $(GT)_3$	182–203	94	6	0.636	0.904	50	40	0.25	Tu-1	HM582834
Tu10	*Tetraneura ulmi*	F: AGTATACGCGTCTCGCCAAC R: GGAGCAAGTCCGATCGTTAT	$(TAA)_3$ TGA $(TAA)_7$	233–248	87	3	0.226	0.253	50	40	0.25		HM582835
Tu11	*Tetraneura ulmi*	F: CGGAGAACGCGTATTGATTT R: CGTGCGCGTGTCAAAGTAT	$(GT)_9$ $(TA)_5$	194–200	89	4	0.396	0.393	50	35	0.25		HM582836
Ac6	*Anoecia corni*	F: CGAGGCATATTCAAAATGTAAGA R: CAGCATTAAACACGAATGCAA	$(AT)_3$ G $(TA)_9$ C $(AT)_2$	148–164	6	2	–	–	45	45	0.25		HM582837
Ac8	*Anoecia corni*	F: AATAATAATTCGTGGCGTTGC R: CGCCGTAGAAGCAAATAATATC	$(ATT)_{10}$	160	4	1	–	–	45	45	0.25		HM582838

N number of tested samples, N_a number of alleles, H_E expected heterozygosity, H_O observed heterozygosity, T_a annealing temperature

near Dejret in 2007 (*Anoecia* spp.). DNA for microsatellite screening was extracted using 200 µl 20%-Chelex® 100 resin (Fluka) (Walsh et al. 1991). PCR-cocktails had a total volume of 10 µl, consisting of 0.8 mM dNTPs, 2 mM $MgCl_2$, 1× PCR buffer, 0.25 U AmpliTaq Gold® DNA Polymerase (Applied Biosystems), 1 µl of DNA template and a varying concentration of primers (Table 1). Several primer pairs were multiplexed in PCR (Table 1). The amplification conditions were 95°C for 5 min, x number of cycles of 95°C for 30 s., T_a for 30 s and 72°C for 30 s (1 min for Gu3, Gu8, Gu9, Gu10 and Gu13) and a final extension of 15 min at 72°C. The respective x and T_a for each primer are listed in Tables 1 and 2.

Amplified fluorescent labeled PCR-products were run on an ABI-PRISM 3130XL (Applied Biosystems) sequencer and chromatograms were analyzed in Genemapper (Applied Biosystems). Expected and observed heterozygosities and deviations from Hardy–Weinberg Equilibrium (HWE) were determined using GENALEX 6.2 (Peakall and Smouse 2006). Occurrence of Linkage Disequilibrium (LD) was assessed using Genepop 4.0 (Rousset 2008).

The fourteen markers developed for *Geoica utricularia* were tested on 5–227 aphids. All markers were polymorphic, with 5.3 alleles per locus on average (Table 1). The four polymorphic markers for *Forda marginata* were tested together with three cross-amplifying markers (Gu6, Gu11, Gu13) on 125–162 aphids yielding 6.0 alleles on average (Tables 1 and 2). The six microsatellite markers for *Tetraneura ulmi* had 3.7 alleles on average in 60–94 tested aphids (Table 1). Observed and expected heterozygosities are given in Tables 1 and 2. Since all species reproduce asexually, deviations from HWE and presence of LD are expected (Ivens et al., in preparation). All loci indeed showed significant deviation from HWE, except for Gu15 in *Geoica utricularia*, Fm4 and Gu11 in *Forda formicaria*, and Tu10 in *Tetraneura ulmi*. In *G. utricularia* the majority of the loci pairs (65%) had significant LD, with most pairs not in LD involving Gu1 and Gu15. All pairs of *T. ulmi* were in LD, except for Tu10-Tu2, Tu10-Tu1, Tu2-Tu11 and Tu1-Tu11. In *F. marginata*, all loci pairs were in LD.

The two primer pairs developed for the genus *Anoecia* amplified across *Anoecia* species but were not extensively tested. We merely report these loci here for future reference.

Cross-amplification was tested for all markers except Gu12 and Fm5 (Table 2), yielding eleven markers that amplified in one or more additional species. Moreover, most markers used (species specific and cross-amplified) for *Forda marginata* were also suitable for the sibling species *Forda formicaria*. The loci Fm3, Fm4, Fm6 and Gu13 proved to be diagnostic for distinguishing between *F. marginata* and *F. formicaria* (Table 2). Three markers from *Pemphigus bursarius* (Pb02 (Miller et al. 2000)) and *P. spyrothecae* (97PS12 and 98PS8 (Johnson et al. 2000)) were tested for cross-amplification in our focal species, but only Pb02 reliably cross-amplified in *Geoica utricularia* (Table 2).

Although we enriched specifically for $(CA)_n$ and $(CT)_n$ repeats, the aphid DNA appeared to be especially AT-rich, including repeats that were suitable for microsatellite

Table 2 Cross-amplifications of microsatellite markers in different species of ant-associated root-aphids

Locus	Cross-amplified species	Size range (bp)	N	N_a	H_E	H_O	T_a (°C)	Nr. of cycles x	Primer concentration (µM)	Genbank accession number
Gu6	*Forda marginata*	151–176	159	5	0.681	0.672	49	40	0.15	HM582818
Gu11	*Forda marginata*	135–147	162	6	0.489	0.234	49	40	0.15	HM582823
Gu13	*Forda marginata*	143–178	159	5	0.430	0.000	45	45	0.15	HM582825
Tu11	*Forda marginata*	–	2	–	–	–	49	40	0.15	HM582836
Fm3	*Forda formicaria*	121	18	1	0.000	0.000	50	40	0.15	HM582828
Fm4	*Forda formicaria*	174–178	18	3	0.495	0.777	50	35	0.15	HM582829
Fm6	*Forda formicaria*	206–291	18	2	0.500	1.000	50	45	0.15	HM582830
Gu6	*Forda formicaria*	151–152	17	2	0.110	0.000	49	40	0.15	HM582818
Gu11	*Forda formicaria*	142–146	18	3	0.439	0.277	49	40	0.15	HM582823
Gu13	*Forda formicaria*	156	19	1	0.000	0.000	45	45	0.15	HM582825
Fm1	*Anoecia corni, A. zirnitsi*	110–134	7	3	–	–	45	45	0.25	HM582827
Tu2	*Anoecia corni, A. zirnitsi*	137–148	3	2	–	–	45	45	0.25	HM582832
Tu11	*Anoecia corni, A. zirnitsi*	69–126	7	5	–	–	45	45	0.25	HM582836
Ac 8	*Anoecia zirnitsi, A. major*	130–146	2	2	–	–	45	45	0.25	HM582838
Pb02[a]	*Geoica utricularia*	118–124	8	2	–	–	50	40	0.20	AF267192

N number of tested samples, N_a number of alleles, H_E expected heterozygosity, H_O observed heterozygosity, T_a annealing temperature

[a] Developed by Miller et al. 2000 for the lettuce root-aphid *Pemphigus bursarius*

design. This observation is in accordance with earlier findings (Weng et al. 2007).

In conclusion, the 26 newly developed microsatellite markers presented here cover a large proportion of the known root-aphid fauna associated with *L. flavus* and other ant species (Heie 1980), and will be useful for detailed studies of the ecology and evolution of this mutualistic association.

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References

Bastolla U, Fortuna MA, Pascual-Garcia A, Ferrera A, Luque B, Bascompte J (2009) The architecture of mutualistic networks minimizes competition and increases biodiversity. Nature 458(7241):1018–1091. doi:10.1038/nature07950

Blomqvist MM, Olff H, Blaauw MB, Bongers T, Van der Putten WH (2000) Interactions between above- and belowground biota: importance for small-scale vegetation mosaics in a grassland ecosystem. Oikos 90(3):582–598

Boomsma JJ, Wright PJ, Brouwer AH (1993) Social structure in the ant *Lasius flavus*—multi-queen nests or multi-nest mounds. Ecol Entomol 18(1):47–53

Dean WRJ, Milton SJ, Klotz S (1997) The role of ant nest-mounds in maintaining small-scale patchiness in dry grasslands in central Germany. Biodivers Conserv 6(9):1293–1307

Godske L (1991) Aphids in nests of *Lasius flavus* F. in Denmark I: faunistic description. Entomol Meddr 59(3):85–89

Godske L (1992) Aphids in nests of *Lasius flavus* F. in Denmark II: population dynamics. Entomol Meddr 60(1):21–26

Heie OE (1980) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. I. Fauna Entomol Scand 9:1–236

Johnson PCD, Llewellyn KS, Amos W (2000) Microsatellite loci for studying clonal mixing, population structure and inbreeding in a social aphid, *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). Mol Ecol 9(9):1445–1446

Lenoir L (2009) Effects of ants on plant diversity in semi-natural grasslands. Arthropod Plant Interact 3(3):163–172. doi:10.1007/s11829-009-9066-7

Miller NJ, Birley AJ, Tatchell GM (2000) Polymorphic microsatellite loci from the lettuce root aphid, *Pemphigus bursarius*. Mol Ecol 9(11):1951–1952

Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6(1):288–295. doi:10.1111/j.1471-8286.2005.01155.x

Pontin AJ (1978) Numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.). Ecol Entomol 3(3):203–207

Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, NJ, pp 365–386

Rütten K, Schulz I, Olek K, Uhl G (2001) Polymorphic microsatellite markers in the spider *Pholcus phalangioides* isolated from a library enriched for CA repeats. Mol Ecol Notes 1(4):255–257

Stachowicz JJ (2001) Mutualism, facilitation, and the structure of ecological communities. Bioscience 51(3):235–246

WallisDeVries MF, Poschlod P, Willems JH (2002) Challenges for the conservation of calcareous grasslands in Northwestern Europe: integrating the requirements of flora and fauna. Biol Conserv 104(3):265–273

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10(4):506–513

Weng Y, Azhaguvel P, Michels GJ, Rudd JC (2007) Cross-species transferability of microsatellite markers from six aphid (Hemiptera: Aphididae) species and their use for evaluating biotypic diversity in two cereal aphids. Insect Mol Biol 16(5):613–622. doi:10.1111/j.1365-2583.2007.00757.x